BIOCHEMICAL GRADIENTS IN THE AXOLOTL GASTRULA

by

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1. INTRODUCTION

Much of the purely factual content of chemical embryology is embodied in statements of the form of (1) and (2):

> (1) The phosphocreatine content of frog's eggs is 1.0×10^{-3} mg/mg nitrogen;
> (2) The anaerobic lactic acid production of ventral ectoderm is 2.1×10^{-4} μg/μg dry weight×hour;

that is, in statements which report the concentration *c* or the activity *a* of some component *x* of a biological system *s* as a function of some reference component *r* of that system.

For many purposes, the choice of an *r* is somewhat arbitrary, and a wide variety of *r*'s is in common use: volume, weight (wet, dry or reduced), cell number, total nitrogen, nucleic acid, and others. In general, it is permissible to employ any of these, but in certain instances it has been recognized that measures of *c* or of *a* thus obtained do not yield unambiguously the kind of information desired. One such instance is the study of chemical and metabolic gradients in the amphibian embryo.

Let us consider, for example, the question whether in the gastrula there is an animal-vegetal gradient in the rate of oxygen uptake. Experiments designed to answer this question usually are run in this manner: specific regions are dissected out along some animal-vegetal meridian, the oxygen uptake O_2 (*s*) of each of these

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explants *s* is measured, and the dry weight or total nitrogen *r* (*s*) of each of these pieces is obtained. The rate of oxygen uptake $Q_{O_2(S)}$ of each region *s*, in terms of some time-unit *t*, is then calculated according to the equation $Q_{O_2(S)} = O_2(s)/r(s) \times t$. When the Q_{O_2} values steadily change from region to region along the animal-vegetal axis, there is said to be a gradient in the rate of oxygen uptake. Such a gradient actually is found in amphibian gastrulae (see review by BOELL (2)) with Q_{O_2} values (*r* = total nitrogen or *r* total dry weight) highest in the animal pole area.

But the adequacy of this general method of establishing gradients in amphibian embryos has been questioned by BARTH (1); and if his views are acceptable, then the correct choice of a reference component is less arbitrary and more difficult. For in the amphibian gastrula there is a yolk gradient along the animal-vegetal axis, with yolk most concentrated at the vegetal pole. Now it is known that yolk has at most a very low respiratory rate. But dry weight or nitrogen is a measure of yolk as well as of "active" protoplasm, and the observed gradient in Q_{O_2}, may, therefore, be interpreted in this way: As regions s are taken nearer and nearer to the vegetal pole, O_2 (*s*) values continuously decrease, since the relative amount of "active" protoplasm in the *s*'s is decreasing, while for explants of similar size (volume) *r*(s) values do not decrease, but rather increase; hence Q_{O_2} steadily decreases. In other words, the respiratory gradient thus measured is a function of the yolk gradient and probably of nothing else. Now, the import of BARTH's discussion is that what we ought to discover is whether or not the "active" protoplasm is respiring at different rates in different parts of the gastrula. This introduces, among others, the problem of finding an *r* which, unlike total nitrogen and dry weight, is a measure of "active" (non-yolk, non-pigment) protoplasm only.

BARTH (1) and BOELL (2) both have carried out procedures designed to meet this requirement; we shall comment on these later (Section 3.1). Our own efforts to discover a measure of "active" protoplasm have resulted in a method for obtaining the non-yolk nitrogen of gastrula explants. Using non-yolk nitrogen (NYN) and also total nitrogen (TN) as reference components we have studied the distribution of alanylglycine peptidase, total saponifiable fat, β-glycerophosphatase and total carbohydrate, in order to compare the two sets of results obtained. Details of the embryological and chemical methods employed are given in the appendices.

2. DISSECTING GASTRULAE

Fig. 1 shows how axolotl (*Ambystoma mexicanum*) gastrulae (HARRISON STAGE 10)[1] were dissected in preparation for chemical analysis.

After removal of the vitelline membrane two cuts were made with glass needles to either side of the dorsal lip in a plane parallel

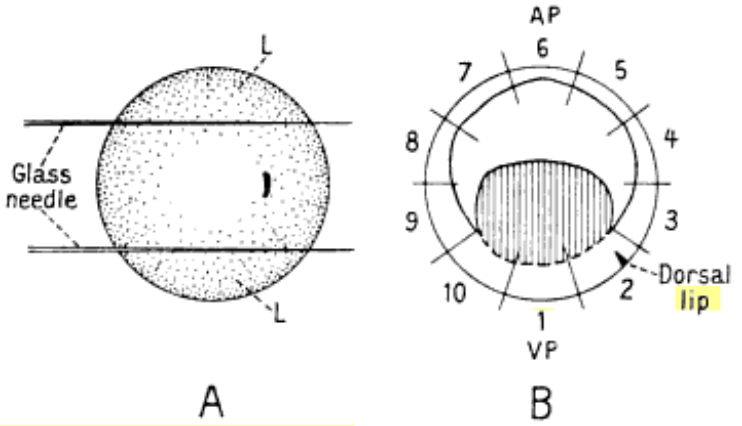

Fig. 1. Dissection of axolotl gastrula. L = lateral piece. VP = vegetal pole. AP = animal pole. A: shows gastrula (vitelline membrane removed) flattened out in operation dish. Vegetal pole uppermost. B: shows the ten pieces into which the meridianal ring of gastrula tissue are cut for analysis.

to the animal-vegetal meridian passing through the dorsal lip (fig. 1, A). The lateral pieces L were discarded. From the ring of tissue remaining the central part of the yolk-cell mass (shaded area, fig. 1, B) was removed and discarded. The remaining tissue was divided into ten pieces of nearly equal length and width (fig. 1, B). Usually about ten gastrulae were dissected for a single experiment and analyses were run on the combined corresponding regions.

3. RESULTS

3.1. Distribution of Non-Yolk Nitrogen (NYN).

GREGG and BALLENTINE (7) found that yolk and pigment granules of frog embryos crushed in 0.65 per cent NaCl in 0.01 molar phosphate buffer at pH 7.4 are stable and are isolable by centri-

[1] See f. inst. R. RUGH: Experimental Embryology, Minneapolis 1948, p. 94. In a few experiments, where sufficient embryos in Stage 10 were not available, an occasional late Stage 9 embryo was used. In these cases the meridian passing through the dorsal lip could not be distinguished.

fugation from the non-yolk, non-pigment portions of the embryos. We have applied this fractionation procedure to gastrula explants, determining the nitrogen present in aliquots taken before (total nitrogen (TN)) and after (non-yolk nitrogen (NYN)) centrifuging.

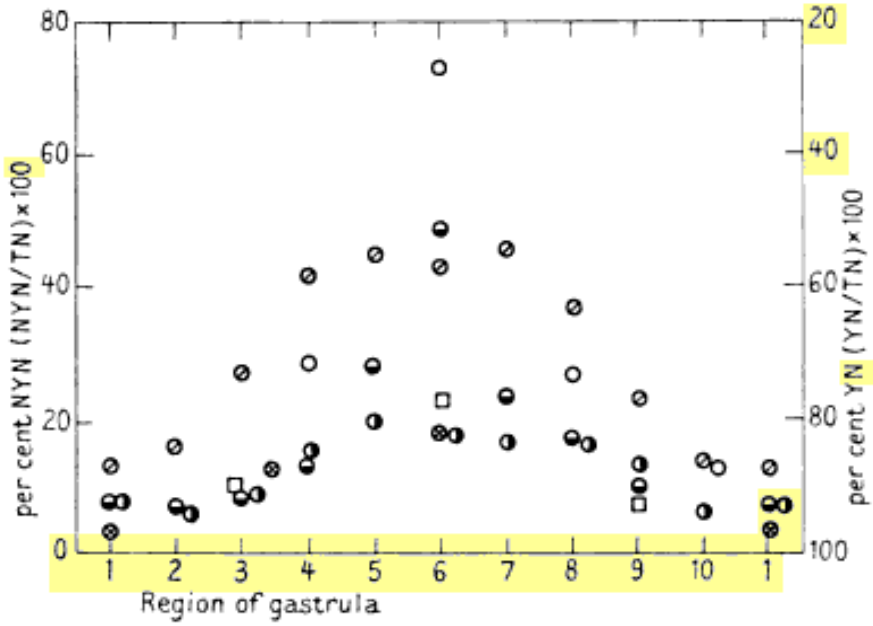

Fig. 2. Distribution of non-yolk nitrogen (*NYN*) and of yolk nitrogen (*YN*) in the axolotl gastrula. Each symbol-type denotes gastrulae from a single salamander female. The same symbols refer to the same gastrulae throughout this paper.

Accordingly we consider (*TN*–*NYN*) = YN to be a measure of yolk[2] present in the explants, while we assume that *NYN* is a measure of "active" protoplasm.

In fig. 2 are plotted the distributions of *NYN* and *YN* in several different batches of gastrulae. On the basis of our hypothesis that *YN* is a measure of yolk and recalling the fact that there is an animal-vegetal yolk gradient in amphibian gastrulae we should expect to find that the per cent *YN* is highest at the vegetal pole (region 1) and lowest at the animal pole (region 6). Clearly this is the case, and to this extent our hypothesis is confirmed. (Incidentally, assuming that yolk is symmetrically distributed, the symmetry of these curves around a maximum at region 6 is evidence that the dissections were made with reasonable accuracy).

[2] Strictly, yolk plus pigment. This should be understood in connection with all the fractionations reported here. The amount of pigment is small relative to the amount of yolk.

The values of YN_V / YN_A (V and A standing for vegetal resp. animal pole) vary, in different batches of embryos, from about 1.2 to about 3.2; average, about 1.5. A comparison of this value with those which can be calculated from the data available on yolk-distribution on other embryos shows (table 1) that they are of the same order of magnitude, though our figure is lower than the rest. We do not know, however, whether the several values given differ because of the different yolk-measures, different reference components, different species, or for some other reason.

Table 1.

Relative yolk content of animal and vegetal pole cells in amphibian gastrulae

Species	yolk component / reference component	% yolk AP	% yolk VP	% yolk VP / % yolk AP	Investigator
Rana pipiens	Wet weight / dry weight	23	49	2.0	BARTH (1)
	volume / dry weight	21	44	2.1	"
Ambystoma punctatum	wet wright / dry weight	24	51	2.1	"
	volume / dry weight	20	46	2.3	"
Ambystoma punctatum	volume / total nitrogen	31—33	66—69	2.1	BOELL (2)
Triturus torosus	volume / volume	19.7	58	2.9	BRAGG (Calc. by BARTH (1))
Ambystoma mexicanum	yolk nitrogen / total nitrogen	60	92	1.5	GREGG and LØVTRUP

The magnitude of the vegetal-animal difference in yolk content may be a function of the amount of yolk present in the whole embryo. For if it is reasonable to suppose that there is a limit to the amount of yolk the vegetal-pole cells can carry, and that a further increase in the relative volume of yolk in the embryo is taken up by cells further toward the animal pole, then in very yolk-rich eggs we should expect to find the yolk gradient to be less steep than in yolk-poor ones. The data in fig. 3, where values of YN_V / YN_A plotted against values of YN (whole embryos) seem to confirm this, but not unambiguously, and decision on this point must await further investigation.

Unfortunately we have no data showing the reproducibility of the experiments for one female, but if the considerable variation between gastrulae from different females in the form of the yolk gradient as well as in its magnitude are real, then our results (see fig. 2; the curves are of different shapes and also of different maximum heights) suggest an important methodological

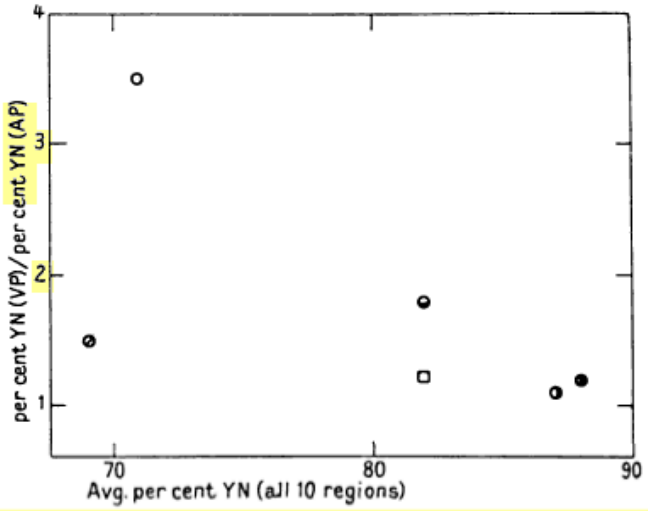

Fig. 3. Relation of amount of yolk nitrogen in whole gastrula to the steepness of the animal-vegetal gradient in yolk nitrogen.

consideration: in all studies in which these factors are of significance, they will have to be determined on the actual material studied; otherwise different sets of data will not be comparable unless the mean values of large numbers of determinations are used which is a less efficient procedure. This requirement has not been met in most previous work on biochemical gradients in amphibian gastrulae. BARTH (1), for example, was the first to attempt to study Q_{O_2} gradients in terms of some measure (r = total dry weight – yolk dry weight) of "active protoplasm", but his determinations of the amounts of active material in different regions of the gastrula were made on explants other than those employed in the actual Q_{O_2} measurements. BOELL (2) also has applied to his Q_{O_2} results corrections derived from determinations of "active protoplasm" (r = total nitrogen × "active" volume in per cent) on gastrulae other than the ones used in the Q_{O_2} measurements. But if variability such as that revealed by fig. 2 is the general rule, then BARTH's and BOELL's procedures do not accomplish the end for which they were designed.

3.2. Distribution of Total Saponifiable Fat.

With references to total nitrogen there is about three times as much saponifiable fat in the animal region of the axolotl gastrula

as in the vegetal region (fig. 4, A); but with reference to non-yolk nitrogen this distribution is altered (fig. 4, B) and there may even be less fat in the animal pole than in the vegetal pole. This is a clear demonstration that the question whether there is a fat gradient in the amphibian gastrula can be answered unambiguously only if a reference component is specified.

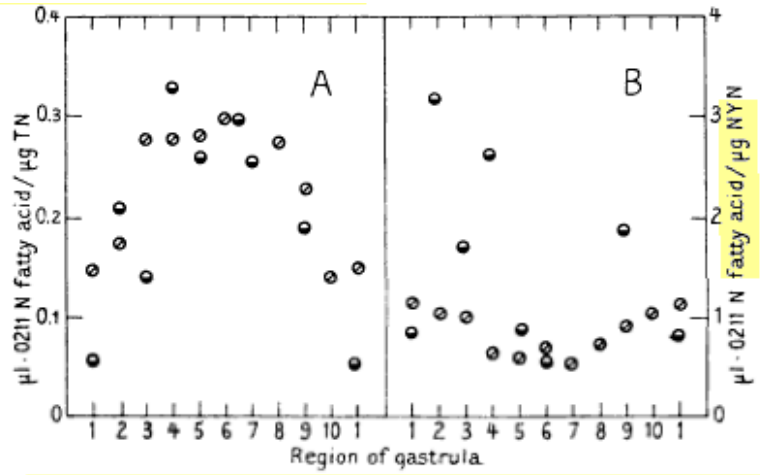

Fig. 4. Distribution of saponifiable fat in the axolotl gastrula, with respect to A: Total nitrogen and B: Non-yolk nitrogen.

But there are still further complications in the study of fat distribution. In yolk (isolated by centrifuging saline breis of unfertilized eggs, washing with saline, dissolving the washed yolk in ten per cent NaCl and dialyzing it until the dialysate no longer yielded a positive silver nitrate test for chloride) the ratio fat/nitrogen[3] is about 0.17. In various batches of whole eggs the ratio fat/nitrogen varies between 0.175 and 0.238. The rough similarity of these two ratios indicates that fat is distributed between yolk and non-yolk portions of the embryo, being perhaps somewhat less concentrated in yolk. This opens up the possibility that there are two fat gradients in the embryo: one in yolk fat, the other in non-yolk fat. To show this, yolk fat and non-yolk fat will have to be studied separately, each in terms of some suitable measure of the embryonic fraction in which it is located.

3.3. Distribution of Total Carbohydrate.

Fig. 5 shows that with respect to total nitrogen there is some six times as much carbohydrate in the animal pole area of the axolotl

[3] μl 0.0211 *N* fatty acids/μg nitrogen.

gastrula as in the area of the vegetal pole. With respect to non-yolk nitrogen the ratio is much less, about two times, but clearly a gradient in carbohydrate is present in the "active" protoplasm (fig. 5, B).

There is no detectable carbohydrate in yolk preparations like those mentioned in Section 3.2. Of course, any dialyzable carbohydrate would be missing from such preparations; but in *Rana pipiens* embryos at least, there is no free glucose, and some three-

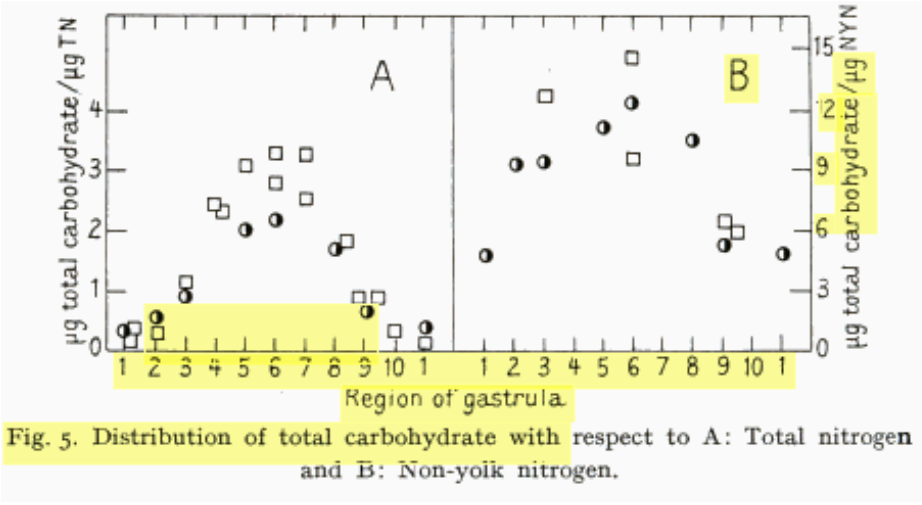

Fig. 5. Distribution of total carbohydrate with respect to A: Total nitrogen and B: Non-yolk nitrogen.

quarters of the total sugar is glycogen (GREGG (6)), which presumably is non-dialyzable. These results together suggest that the carbohydrate present in amphibian embryos is located in non-yolk parts.

3.4. Distribution of Alanylglycine Peptidase.

In the axolotl gastrula there is an animal-vegetal gradient in alanylglycine peptidase with respect to both total nitrogen and non-yolk nitrogen (fig. 6).

PICKFORD (13) has studied the spatial distribution of alanyl-glycine peptidase (r = dry wt. of material extractable in 30% glycerol = *G*) in *Ambystoma punctatum* gastrulae. Her data lead her to conclude that peptidase activity is a linear function of *G* (which she takes to be a measure of non-yolk material). A logical consequence of this conclusion is that "there are no significant differences in the peptidase content of the soluble proteins from different regions of the gastrula", i.e., that there is no gradient in peptidase activity (with respect to *G*) in the gastrula. This is in apparent disagreement with our present results.

Several possible reasons for this discrepancy can be suggested. (a) *NYN* and *G* may not be interchangeable reference components; that is, there may be a gradient in peptidase with respect to *NYN* but not with respect to *G*. In this case PICKFORD'S and our results are not contradictory at all, but simply reflect a difference in the choice of a reference component. (b) 30 % glycerol

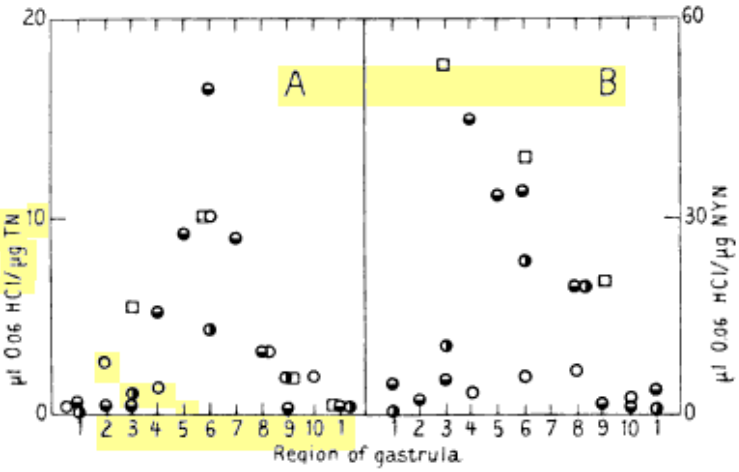

Fig. 6. Distribution of peptidase in the axolotl gastrula with respect to A: Total nitrogen and B: Non-yolk nitrogen.
◒ peptidase determination on whole brei only
◑ peptidase determinations on extract (brei supernatant) only
□ and ○ in A: peptidase determination on whole brei, in B: peptidase determination on extract.
○ is atypical in showing only a slight peptidase gradient with respect to *NYN*.

(as used by PICKFORD) and 0.65% saline-phosphate (as used by us, see appendix B) may not be interchangeable extract- ants for peptidase. The addition of 30% glycerol to our saline-phosphate makes no difference in the amount of peptidase extracted (fig. 7); but the presence of salt here seems important, since PICKFORD reported that 0.01 *M* phosphate extracts were inactive. However, we have not compared the activity of plain 30% glycerol extracts with that of saline-phosphate extracts, and we cannot decide whether our data and PICKFORD's differ for this reason. (c) Peptidase activity may not be a linear function of *G*, as PICKFORD believes. There is sufficient scatter in the results plotted in her fig. 7 to make it at least possible that the relation is non-linear. In this case, it follows that there is a gradient of

some kind present. It would be instructive to be able to plot PICKFORD'S peptidase/*G* ratios against some kind of spatial coordinate which shows precisely their positions in the gastrula, but this cannot be done from the published account of her work. (d) Finally, if (a), (b) and (c) should fail as explanations, there is always the possibility that there are species differences in pepti-

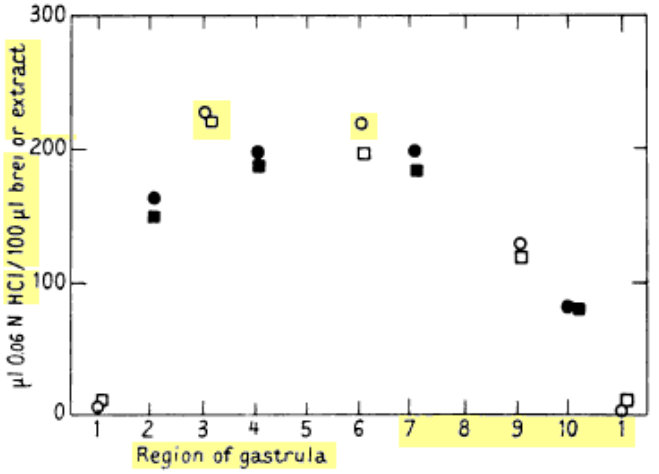

Fig. 7. ■ glycerol-saline-phosphate brei supernatant (extract)
□ saline-phosphate extract
● glycerol-saline-phosphate whole brei
○ saline-phosphate whole brei.
All explants from the same set of gastrulae. Note (1) that about the same amounts of enzyme are extracted whether or not glycerol is present, and (2) that whole brei and extract show about the same peptidase activity, thus demonstrating that yolk contains little or no peptidase.

dase distribution; but the likelihood of this cannot be evaluated in the absence of more information.

Incidentally, our results (fig. 7) agree with PICKFORD'S in showing that yolk has little or no peptidase activity.

3.5. Distribution of alkaline β*-glycerophosphatase.*

With respect to total nitrogen, there is about six times as much alkaline phosphatase activity in the animal pole area as in the vegetal pole area of axolotl gastrulae (fig. 8, A). Dorsal regions (2-5) seem to be more active than corresponding ventral ones, but we

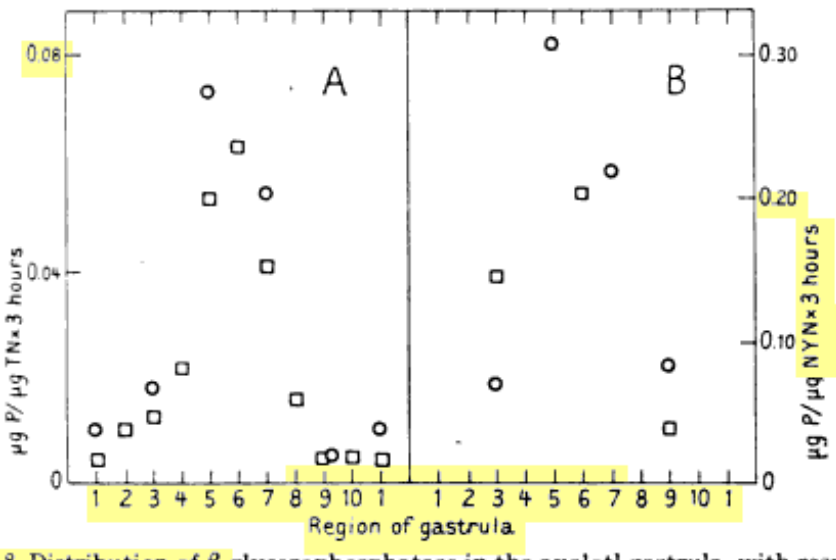

Fig. 8. Distribution of β-glycerophosphatase in the axolotl gastrula, with respect to A: Total nitrogen and B: Non-yolk nitrogen.

shall not consider this as having been demonstrated unless it is supported by much more extensive work.

There is also a sharp animal-vegetal gradient in this enzyme with respect to *NYN* in the gastrulae investigated (fig. 8, B).

3.6. Distribution of Oxygen Uptake.

The egg-laying season was just ending when we began this part of the work, and we are able to report (as a matter of general interest) only the results of measurements on a single gastrula (fig. 9). In this gastrula there is a difference of some 11-12 times between the greater respiration of the animal pole explant (6) and the smaller respiration of the vegetal pole explant (1) (r = total nitrogen). Determinations of *NYN* were not made, and we cannot, therefore, suggest what the distribution of respiration in terms of this reference component would have been.

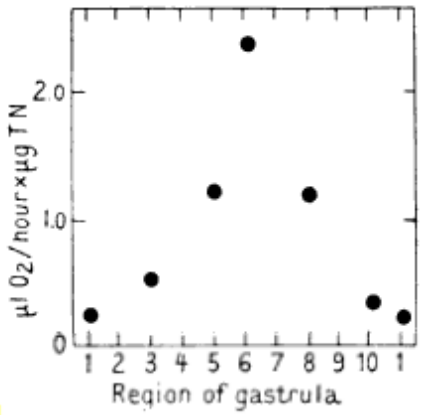

Fig. 9. Distribution of oxygen uptake in a single axolotl gastrula (from salamander ○).

4. DISCUSSION

From the foregoing, it should be clear that an explicit statement about a gradient in any biological system *s* must mention at least the following:

(a) A component *x* of *s*, which is said to be distributed.

(b) At least two spatial positions in *s* between which there is said to be a distribution of (gradient in) *x*.

(c) A reference component *r*, in terms of which the concentrations (or activities) of *x* are expressed.

The literature on gradients contains several examples where statements with respect to (b) and (c) are ambiguous, thus making the interpretation of the results difficult . Some few cases may be mentioned:

Failure to specify spatial coordinates. Examples of this ambiguity are common in the early literature of amphibian chemical embryology. BOELL (2), in reviewing the reasons for disagreement about respiratory gradients in the amphibian gastrula, says that "Perhaps the factor of greatest importance has been the failure of various workers to use corresponding areas of dorsal lip and ventral ectoderm for comparison." PICKFORD'S interesting work, as we have seen, would have been much more interpretable had she indicated the precise anatomical location from which her material was taken.

Failure to obtain a quantitative measure of r. Again some of the early work on the distribution of respiratory activity in amphibian embryos suffers from this defect, since the respiration of explants of indefinitely known and variable size was reported (cf. BOELL (2)), and it is impossible in some cases to decide whether the reported respiratory variations are due simply to this factor alone.

These two difficulties are well-known and have often been mentioned. There is a third, however, which has received relatively little attention. It concerns the comparison of results based on entirely different reference components. For example, BOELL'S (2) table 1 demonstrates the great variance in the results of work by different investigators on the respiratory rates of dorsal lip and ventral ectoderm in the amphibian gastrula. Some of these results were based on dry weight, others on wet weight, others on total nitrogen, and one on an unspecified reference component; yet in his discussion of probable reasons for the variance, BOELL does not mention the

possibility that it is connected with variation in the reference components chosen. It is obvious, however, that genuine comparisons cannot be made until interconversion factors are known for these various reference components for each region of the gastrula.

While we cannot make accurate comparisons between results, obtained by using different reference components, *r*, it is nevertheless possible to arrange some of the most commonly used *r*'s with regard to their relative value as functions of "active" protoplasm.

In the following scheme are included the reference components: volume, *V*; wet weight $W = V \bullet \varphi$, where φ is the density; dry weight, $DW = W \bullet x$, x being the percentage of dry matter; reduced weight, $RW = V(\varphi - 1)$; and total nitrogen, *TN*. In establishing the scheme we assume that we have two explants, one from the animal, *A*, and one from the vegetal pole, *V*, containing the same volume, *v*, of "active" protoplasm, i.e. the volume not occupied by yolk: $v_A = v_V$. It is possible to state the following relations:

$$1 = \frac{v_V}{v_A} \underset{[1]}{<} \frac{V_V}{V_A} \underset{[2]}{<} \frac{W_V}{W_A} \overset{[3]}{<} \frac{DW_V}{DW_A} \overset{[6]}{<} \frac{TN_V}{TN_A}.$$

$$\underset{[4]}{<} \frac{RW_V}{RW_A} \underset{[5]}{<}$$

Since we know that the volume percentage of yolk in the vegetal region (BARTH (1), BOELL (2)), is greater than in the animal region, and since the non-yolk volumes, *v*, are assumed equal, the total volumes, *V*, must satisfy [1]. Again, the wet weight ratio is obtained by multiplication of the volume ratio by $\varphi_V/\varphi_A > 1$ (BARTH (1)), and accordingly [2] is established. Since the percentage of dry substance, *x*, must be greater in the vegetal than in the animal pole (higher density), and the *DW* ratio is obtained by multiplying wet weight ratio by $x_V/x_A > 1$, [3] is proved. From $\varphi_V > \varphi_A$ follows $\varphi_V/\varphi_A < \varphi_V - 1/\varphi_A - 1$ $(\varphi > 1)$, from which [4] is derived. To establish [5] it must be remembered that *RW* essentially is a measure of proteins (assumed proportional to *TN*) and carbohydrates (see HOLTER and ZEUTHEN (8)), and since the relative amount of carbohydrates is higher in the animal than in the vegetal pole (compare fig. 5) it follows that the *RW* ratio must be smaller than the *TN* ratio. In equation [6] holds good if TN_V / TN_A is greater than (carbohydrate + fat)$_V$ / (carbohydrate + fat)$_A$, which is the case according to the results obtained here.

The reference component extensively used in this paper, $r = NYN$ or extractable nitrogen, cannot be included the scheme. We have been assuming that NYN_V / NYN_A is equivalent to v_V / v_A, but no proof could be offered.

The relations established above enable us to predict in which direction results, obtained by using different r's, will vary. If we want to compare the activity A (enzymatic, respiratory etc.) per unit r in animal and vegetal regions the following expression must be found: $C = \frac{A_A}{r_A} / \frac{A_V}{r_V} = \frac{A_A}{A_V} \bullet \frac{r_V}{r_A}$. It is seen from this that the greater the value of r_V / r_A, the greater value of C will be found. F. inst. it is to be expected that comparisons made with $r = TN$ should give higher values than if $r = DW$ is used. Unfortunately, we could not find data in the literature which can be used to test this. BOELL (2) has done some experiments concerning the oxygen uptake of explants of *Ambystoma punctatum* and finds a ratio of 4-5 between maximum and minimum respiration, using $r = DW$. This value is definitely lower than the one found by us (11-12), using $r = TN$, but since we have done only one experiment and since the explants used by us were smaller ($\sim\frac{1}{20}$ of the circumference) it is not possible with certainty to ascribe the diverging results to the different reference components.

Finally, species-variation should always be considered when comparisons are being made. We do not believe that it will ever be possible to adopt a standard reference component suitable for all types of investigations (see, however, the recent interesting paper of DAVIDSON and LESLIE (5) who propose desoxyribosenucleic acid as a theoretically advantageous reference component); but the establishment of useful generalizations might be greatly enhanced by the adoption of a standard reference component in terms of which the results of all investigations could be expressed by using conversion factors routinely supplied by the investigators. In this manner, results obtained by different workers would become interconvertible and hence fruitfully comparable.

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APPENDIX A

Embryological Methods

Obtaining and rearing embryos: Adult *Ambystoma mexicanum* were kept in the laboratory in individual earthenware jars. When fertilized eggs were needed, a male and a female were put together in an enameled metal tank of tap water. After the onset of egg-laying, the eggs were collected at frequent intervals and spread out thinly in finger bowls in tap water, and allowed to develop. When necessary, development was slowed by placing the embryos in a refrigerator at abouth 10 °C.

Explanting: All operations were done in full strength Holt- freter's solution without bicarbonate. Healing of the explants was allowed to take place for 1-4 hours in this solution. Explants which failed to heal were not used.

Making brei: Cell-free breis were made by crushing the explants in narrow glass tubes with a ball-tipped glass rod which closely fitted the tubes. Usually there were 10 explants per 100 μl 0.65 per cent NaCl in 0.01 *M* phosphate buffer at pH 7.2–7.4, but this proportion was varied somewhat to meet momentary needs.

Centrifuging: Yolk and pigment granules were sedimented by centrifuging the brei-tubes for about 15 minutes at about 4000–5000 × g.

APPENDIX B

Chemical Methods

Kjeldahl nitrogen: The method of BRÜEL et al. (4), was somewhat modified as follows: (1) Digestion: Material containing up to 7 μg *N* digested in 25 mm × 2–3 mm tubes (3 μl conc. H_2SO_4 and 6 μl blue mixture) on a sand bath. (2) Transfer of digest to distillation tubes: Instead of a paraffin block, a glass slide coated with

silicone was used. (3) Distillation: Into 6.9 µl 0.1 N H_2SO_4. (4) Titration: After adding 17 µl phosphate indicator mixture (seven times more concentrated than in original method) titrated in usual manner with 0.09 *N* HCl containing brom cresol green. (5) Recovery and reproducibility: Test material, casein. On test run (9 determinations) the recovery was 93 per cent of theoretical, with a standard deviation of 0.06 µg *N*.

Saponifiable Fatty Acids: The method of SCHMIDT-NIELSEN (14, 15) was used without modification. According to BOYD (3), the saponifiable fats constitute nearly all of the total fat in ovarian eggs of *Rana pipiens*. In a test run (6 determinations) on sample containing 12.1 µg oleic acid, 12.8 ± 0.72 µg were recovered.

Alanylglycine peptidase (substrate: D, L-ananylglycine): The method of LINDERSTRØM-LANG and HOLTER (10) was used. Alkaline phosphatase (substrate: β-glycerophosphate): The method of KRUGELIS (9) was used. This colorimetric method (range 4×10^{-3} to 4×10^{-2} µg *P*) is based on the reduction of phosphomolybdic acid by stannous chloride.

Total carbohydrate: The method used for determination of total carbohydrate was a micro-modification of the SOMOGYI method (16), including the changes made by GREGG (6).

The procedure was: 83 µl embryo extract (5–10 µg glucose), 55 µl 6% HCl and a magnetic stirrer ("flea") were added to a thin-walled glass tube. The tube was sealed and the content mixed. The tube was placed in a water bath (100 °C) for three hours, after which the liquid on the walls was centrifuged down and the tube opened. Now 20 µl 5% sodium tungstate solution was added, the content stirred, and the tubes, closed with rubber caps, centrifuged. An aliqout of 128 µl was transferred to a micro vessel, neutralized with 2 µl NaOH solution (about 35%, adjusted so that the resulting solution was slightly alkaline), and mixed with a magnetic stirrer. Now three aliquots of 40 µl were transferred to three silicone coated micro vessels, and to each added 50 µl cupper reagent solution (containing 28 g anhydrous disodium phosphate, 100 ml *N* NaOH, 40 g Seignette salt, 8 g cupric sulfate and 180 g anhydrous sodium sulfate, 12.5 ml 1 *N* potassium iodate, and 5 g potassium iodide). The solutions were mixed with a flea, the tubes closed with caps and heated in a water bath (100 °C) for ten minutes. After cooling, a seal consisting of 30 µl 0.1% sodium starch glycollate (PEAT, BOURNE, and THROWER (12)) solution was placed in the upper end

of the reaction vessel. The solution was acidified by adding 20 μl 5 N H_2SO_4 through the seal. The iodine evolved was titrated with 0.02 N thiosulfate, first through the starch seal, which was united with the solution in the bottom of the vessel , when the major part of thiosulfate had been added (LINDERSTRØM-LANG and HOLTER (11)). The standard curve was linear, corresponding to 1.52 μl 0.20 N thiosulfate per μg glucose. The standard deviation calculated for a number of determinations was 0.20 μl 0.20 N thiosulfate, i.e. 0.13 μg glucose. This rather high standard deviation was mainly due to that, from time to time, one determination deviated very much from the two others, without it being possible to explain the deviation. Due to this the copper method does not seem to be advantageous in micro modification and the use of it has later been abandoned.

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Rings of tissue from axolotl gastrulae cut parallel to the animal-vegetal axis, were cut into 10 pieces each. These pieces were used for determination of total nitrogen (*TN*), and for analysis of the distr. of non-yolk nitrogen (*NYN*), saponifiable fat (*SF*), total carbohydrate, dipeptidase, alkaline phosphatase, and oxygen uptake. A gradient with respect to *TN*, having its maximum at the animal pole, was found in all cases. Since the yolk gradient present in the eggs makes the use of *TN* as reference inexpedient, a better picture of the biochemical distr. was sought by using *NYN* instead of *TN*. This procedure did not obliterate the animal-vegetal gradients obtained except in the case of *SF*, which is evenly distr. in the gastrula with respect to *NYN*. The results were compared with similar results obtained by other authors.